Applications of Synchrotron Infrared Microspectroscopy to the Study of Inorganic-Organic Interactions at the Bacterial-Mineral Interface

H-Y. N. Holman, D.L. Perry,
M.C. Martin, and W.R. McKinney

Reprinted from Materials Research Society
Symposium Proceedings Volume 524,
Applications of Synchrotron Radiation Techniques to Materials Science IV,
S.M. Mini, S.R. Stock, D.L. Perry,
and L.J. Terminello, editors.

APPLICATIONS OF SYNCHROTRON INFRARED MICROSPECTROSCOPY TO THE STUDY OF INORGANIC-ORGANIC INTERACTIONS AT THE BACTERIAL- MINERAL INTERFACE

HOI-YING N. HOLMAN*, DALE L. PERRY, MICHAEL C. MARTIN, AND WAYNE R. McKINNEY, Lawrence Berkeley National Laboratory, University of California, Berkeley, CA 94720, hyholman@lbl.gov

ABSTRACT

Synchrotron microspectroscopy has been used to study the inorganic-organic interactions in the mid-infrared region (4000-400 cm^{-1}) as *Arthrobacter oxydans* attach themselves to magnetite surfaces. Relative band intensities and band intensity ratios for functional groups of organically-derived biological molecules that are inherent to the experimental system are discussed. The molecular components as they are perturbed by interactions with water, dichromate and chromate metal ions on the mineral surfaces are investigated. Mapping of the spectral markers for the inorganic-organic interactions at the biological-mineral interfaces is presented and discussed. Comparative analyses of the synchrotron infrared microspectra suggest that the bacterial-chromium interactions depend on the solubility and toxicity of the chromium compounds.

INTRODUCTION

Pollution of subsurface geologic zones by heavy metals and the possibility of using intrinsic endolithic (rock/mineral-inhabiting) bacteria to either detoxify or immobilize the pollutants have stimulated new interests in the exploration of the bacterial responses to metal ions in geologic environments [1-2]. It is widely believed that the inorganic-organic interactions at the biological-mineral interfaces, which mostly arise from bacterial defense mechanisms, can change the effectiveness of bacteria to remediate metal ions in the immediate vicinity [3]. In the case of chromium, it is generally accepted that the enzymatic reduction of hexavalent chromium Cr(VI) (chromate) is one defense mechanism employed by bacteria living in the chromate-contaminated environment [4]. Most of the previous studies on the interactions between bacteria and metal ions were conducted by means of batch or column experiments [4-5], which can only globally address the questions of whether or not the bacteria are interacting with the metal ions. To evaluate if the mechanisms of the interaction exist at the biological-mineral interfaces, one needs to examine and monitor, nondestructively, the structural changes of molecules as the reactions take place *in situ*.

In this study we present the use of synchrotron-infrared absorption-reflectance microspectroscopy as a chemical/biological microprobe to study — *in situ* and nondestructively — the responses of bacteria to Cr(VI) at the biological-mineral interfaces. Specifically, we investigated if the synchrotron microprobe could successfully (1) monitor the inorganic-organic interactions and (2) illustrate the highly localized differences in the progress of the interactions and the structural details of clusters of living-bacteria in minerals that inherently have low infrared reflective surfaces. *Arthrobacter oxydans* and magnetite, the bacteria and mineral used in this study, are representative of those commonly encountered in the subsurface [6,7]. Results from the synchrotron infrared microspectroscopy analysis provide new insights into factors that control bacterial defense mechanisms and further our understanding of the bacteria's capability to remediate metal contaminants in geologic environments.

EXPERIMENTAL

Chemicals

All chemicals were purchased from Aldrich (USA). They were of A.C.S. reagent grades with a purity of at least 99%. Cr(VI) in this study was derived from two different chromium compounds:

Mat. Res. Soc. Symp. Proc. Vol. 524 © 1998 Materials Research Society

potassium chromate and dichromate (from Aldrich, USA). Their aqueous solutions were prepared at pH 7.4 (physiologic pH) in phosphate buffer solutions. Potassium chromate and dichromate were selected as our model compounds to represent a high solubility/toxicity and a low solubility/toxicity chromate compound at the physiologic pH. Toluene vapor was used as a carbon source for the bacteria when needed.

Bacteria and Minerals

A. oxydans were isolated from rock cores collected from a subsurface rock vadose zone at a depth of 66-70 m at a site within the extensive Columbia basalt flow in southeastern Idaho as previously described [8]. *A. oxydans* were maintained in a filter-sterile liquid growth medium that consisted of 40% of basalt extract solution and 60% of distilled and deionized water. The basalt extract solution was prepared by mixing vigorously and heating (without boiling) for one-hour 500 g of basalt grains in 1 liter of distilled, deionized water. The liquid was strained through a double-layer cheesecloth and filter sterilized with 0.2 μm cellulose acetate/cellulose nitrate mixed esters (CA/CN) filters.

Magnetite mineral samples were obtained commercially from Minerals Unlimited (Ridgecrest, CA). Thin magnetite specimens were prepared by cleaving fragments off the micro-fissiles. The specimen surfaces were cleaned by sonification in deionized and organic-free water for fifteen minutes and sterilized by UV-irradiation for twenty minutes.

Inorganic-Organic Interaction Experiments

To generate the experimental conditions for studying the inorganic-organic interactions at the bacterial-mineral interfaces, *A. oxydans* were introduced onto the magnetite surfaces. This procedure involved first capturing the bacteria from the growth liquid medium onto the 0.2 μm CA/CN filters and printing onto the magnetite surfaces using a technique similar to the agar printing-off procedure described in [9]. Five hours after the bacterial attachment to the magnetite surfaces, they were exposed to 10 ppm of either chromate or dichromate solutions. The temporal variation and the spatial distribution of the inorganic-organic interactions on the biological-mineral surfaces were measured spectroscopically with the infrared microprobe at the Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory. For each measurement at a given location, 128 spectra were co-added at a spectral resolution of 4 cm^{-1}. All infrared spectral characteristics were interpreted according to well-documented spectral research literature [12-15].

Instrumentation

The progress and the spatial distribution of chromium-bacteria interactions at the biological-magnetite interface was monitored using the infrared microspectroscopy Beamline 1.4.3 at the Advanced Light Source (ALS), Lawrence Berkeley National Laboratory as previously described [10,11]. The infrared microprobe uses a synchrotron source that has much higher brightness than a conventional thermal IR source. The synchrotron light is input into a Nicolet Magna 760 FTIR bench, then passed through a Nic-Plan IR microscope. As detailed elsewhere [10], the experimental spot size of the unmasked synchrotron beam focused through an infrared microscope is 10 microns, a 100 fold smaller than is possible with an internal thermal source. This experimental procedure is non-destructive to the biological materials being studied.

RESULTS AND DISCUSSION

Dichromate-Bacteria Interactions

In the first study *A. oxydans* was exposed to a 10 ppm dichromate $(Cr_2O_7)^=$ solution. The dichromate-bacteria interactions on the magnetite surfaces were monitored for two days. Figure 1 shows

the synchrotron FTIR absorption microspectra in the region 1750-650 cm^{-1} for the living *A. oxydans* cells before their exposure to the 10-ppm $(Cr_2O_7)^=$ solution. The global infrared spectral features for the living cells prior to the bacterial exposure to $(Cr_2O_7)^=$ are consistent with those reported in the literature [12]. All absorption bands are related to the vibrations of the functional groups of the biomolecules of bacteria cell walls. The prominent absorption envelopes in the 1695-1620 cm^{-1} and 1515-1570 cm^{-1} regions arise from the C=O amide vibrations of the peptide linkage in the bacteria protein Amide I and II systems. The absorption band between 1250 and 1200 cm^{-1} arises from the P=O double-bond asymmetric stretching mode of phosphodiester, free phosphate, and monoester phosphate functional groups of the polysaccharide backbone structures. The complex sequence of peaks in the 1200-900 cm^{-1} region are essentially due to C-O-C and C-O-P stretching vibrations of mostly oligo- and polysaccharidic components.

Two additional absorption peaks at 906 and 884 cm^{-1} were detected after the $(Cr_2O_7)^=$ solution was introduced into the biological-mineral system. The vibrational frequencies of these peaks are the same as those reported for $(Cr_2O_7)^=$ in the literature [13], this confirms the presence of $(Cr_2O_7)^=$ in our system. The shifting of the 1640 cm^{-1} band to a higher frequency of 1650 cm^{-1} after the bacterial exposure to $(Cr_2O_7)^=$ implies that the bacterial protein Amide I structure increased its "randomness" or became more disordered. The width of the Amide I vibration band, which is proportional to the amount of water bound to the albumin molecules [12], remained constant and did not accompany the structural change of the bacterial protein. The occurrence of a prominent new peak at 935 cm^{-1} shortly after the exposure to $(Cr_2O_7)^=$ is probably due to the coupling of C-O-Cr and ring C-O-C [14]. The shifting of the 935 cm^{-1} band to 950 cm^{-1} with time in a biological system has not been reported in the literature. We speculate that it is possibly due to the weakening in the strength of hydrogen bonding in the presence of $(Cr_2O_7)^=$. This needs to be investigated. The increase in its relative absorption intensity with time implies the presence of the $(Cr_2O_7)^=$ stimulated the production of polysaccharide. Similar phenomena have been reported for other metal ions in the literature [14].

Chromate-Bacteria Interactions

This study was carried out by exposing *A. oxydans* to a 10 ppm chromate $(CrO_4)^=$ solution. The chromate-bacteria interactions on the magnetite surfaces were also monitored for two days. Figure 2(a,b) shows the synchrotron FTIR absorption microspectra in the region 1750-650 cm^{-1} for the living *A. oxydans* cells before their exposure to the 10-ppm $(CrO_4)^=$ solution. Their global infrared spectral features prior to the exposure in this experiment again are consistent with those reported in the literature [12].

An additional absorption peak at 846 cm^{-1} (Figures 2b) was detected after the $(CrO_4)^=$ solution was introduced into the biological-mineral system. The vibrational frequency 846 cm^{-1} has been assigned to $(CrO_4)^=$ in the literature [13], thus confirming the presence of chromate $(CrO_4)^=$ in the biological-mineral system. After contact with *A. oxydans* cells, the $(CrO_4)^=$ peak shifted to a lower frequency with time (Figure 2b), which arose from a decrease in the oxidation state of the chromium [15], in the presence of the bacteria.

The spectral characteristics in Figure 2a illustrate that the biomolecules of *A. oxydans* interacted with $(CrO_4)^=$ differently. In addition to a shift of the Amide I vibrational band from 1640 cm^{-1} to a higher frequency, a narrowing of the bandwidth of the Amide I vibration band was also detected. This implies that the interaction between $(CrO_4)^=$ and biomolecules could decrease the amount of water bound to the albumin molecules and resulted in an increase in the secondary protein structure to unordered structure. The appearance of multiplets in the Amide I and Amide II absorption envelopes was probably caused by the degradation and denaturing of the proteins, most likely caused by the decrease in water in the cells. The progressive decrease in the relative intensity of absorption bands for functional groups of other biomolecules in region below 1460 cm^{-1} was associated with the observed microscopic change of *A. oxydans* cells from the initial rods to their stressed form cystites after their exposure to $(CrO_4)^=$.

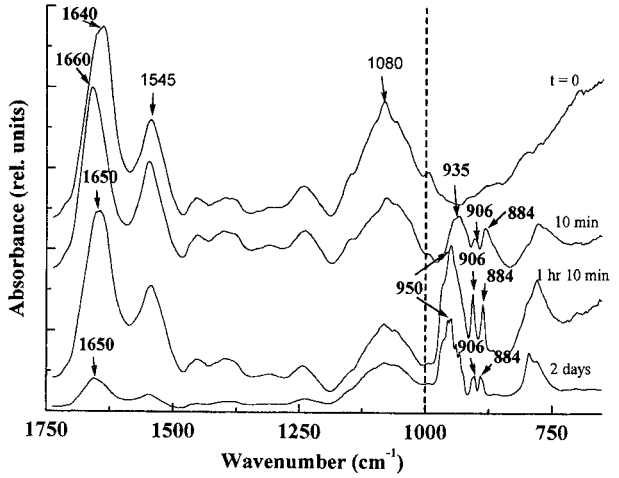

Fig. 1. Synchrotron FTIR absorption microspectra in the region 1750-650 cm^{-1} for the living *A. oxydans* cells before and after their exposure to the 10-ppm $(Cr_2O_7)^{=}$ solution.

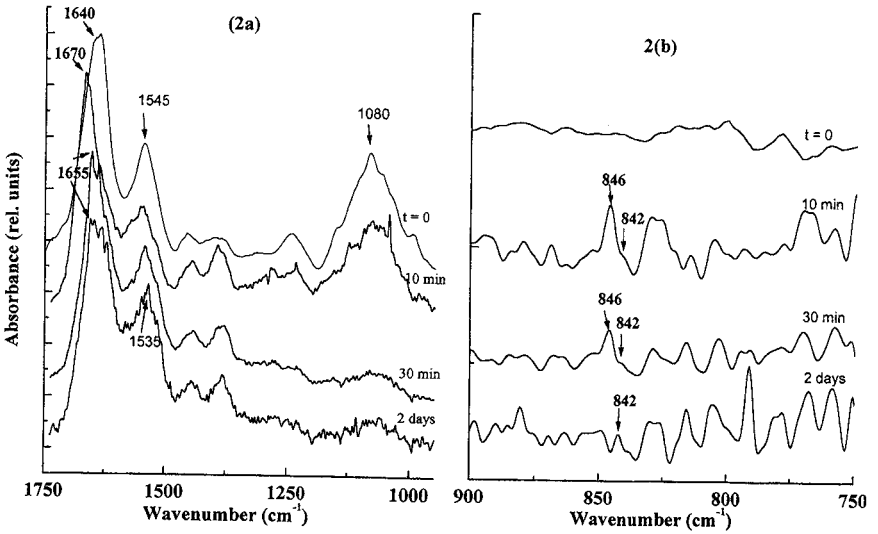

Fig. 2. Synchrotron FTIR absorption microspectra in the region (a) 1750-1000 cm^{-1} (b) 1000-650 cm^{-1} for the living *A. oxydans* cells before and after their exposure to the 10-ppm $(CrO_4)^{=}$ solution.

Mappings of Chromate-Bacteria Interactions on Magnetite

This study was carried out by first exposing the magnetite surfaces to a 80-ppm toluene vapor for five hours prior to the introduction of *A. oxydans*. This additional experimental step was to provide *A. oxydans*, a toluene degrader [16], a carbon source so that the experiment could last for a longer period of time. Figure 3 shows a representative synchrotron FTIR absorption microspectrum in the region 1750-650 cm^{-1} for the surface of our biological-mineral system after exposure to a 10-ppm chromate solution in the presence of toluene as a carbon source.

The spatial variation of chromate-bacteria interactions on the magnetite surfaces were monitored five days after the initial exposure to the 80-ppm toluene vapor and a subsequent exposure to a 10-ppm chromate solution. Figure 4 shows the 3-D maps of the spatial distribution of the vibrational modes associated with *A. oxydans* (1665 cm^{-1}), chromate $(CrO_4)^{=}$ (846 cm^{-1}), and the toluene (728 cm^{-1}) on a magnetite surface. We observed that at the location of the *A. oxydans* (peak in the leftmost plot) there is a significant decrease in the amount of both chromate and toluene (right two plots).

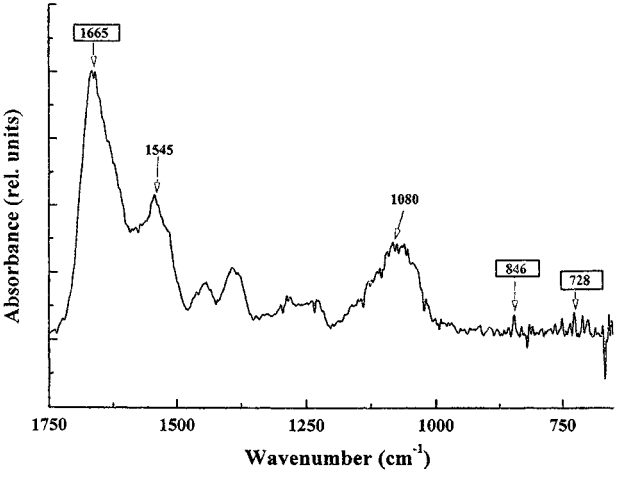

Figure 3. A synchrotron FTIR absorption microspectrum in the region 1750-650 cm^{-1} for the surface of our biological-mineral system after exposure to a 10-ppm chromate solution in the presence of toluene vapor.

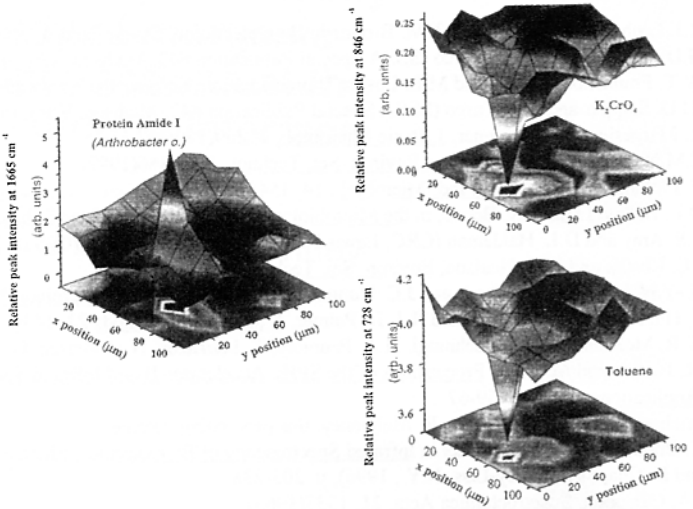

Figure 4. 3-D maps of the spatial distribution of the vibrational modes associated with *Arthrobacter o.* (1665 cm^{-1}), chromate $(CrO_4)^{=}$ (846 cm^{-1}), and the toluene (728 cm^{-1}) on a magnetite surface.

CONCLUSIONS

The above results demonstrate that the synchrotron microprobe could nondestructively monitor the *in situ* inorganic-organic interactions at the biological-mineral interfaces. It also illustrates the highly localized differences in the progress of the interactions and the structural details of clusters of living-bacteria in minerals. Comparative analyses of the synchrotron infrared microspectra suggest that the mode of bacterial transformation of the hexavalent chromium Cr(VI) depends on the solubility and toxicity of the chromium compounds.

ACKNOWLEDGMENTS

We thank Dr. S. Goldman at the University of California for reviewing the manuscript. This work was performed with support by the Directors, Office of Energy Research, Offices of Health and Environmental Sciences, Biological and Environmental Research Program and Basic Energy Sciences, Materials Science Division, of the United States Department of Energy under Contract No. DE-AC03-76SF00098.

REFERENCES

1. R.J. Lenhard, R.S. Skeen and T.M. Brouns in Bioremediation: Science and Applications, edited by H.D. Skipper and R.F. Turco (SSSA Special Publication #43, Madison, Wis., 1995), p.157-172.
2. W.T. Frankenberger, Jr. and M.E. Losi in Bioremediation: Science and Applications, edited by H.D. Skipper and R.F. Turco (SSSA Special Publication #43, Madison, Wis., 1995), p.173-210.
3. R. Margesin and F. Schinner, J. Basic Microbiol., **4**, 269(1996).
4. E.M.N. Chirwa and Y.T. Wang, Environ. Sci. Technol., **31**, 1446(1997).
5. Y.T. Wang and H. Shen, J. Ind. Microbiol., **14**, 154(1995)
6. D.L. Balkwill and D.R. Boone, in the Microbiology of the Terrestrial Deep Subsurface, edited by P.S. Amy and D.L. Haldeman (CRC, Lewis Publishers, N.Y., 1997), p. 105-118.
7. J.E. Kostka and K.H. Nealson, Environ. Sci. Technol., **29**, 2535(1995)
8. H.-Y.N. Holman, D.L. Perry and J.C. Hunter-Cevera, submitted to J. Microbiol. Methods, 1998.
9. P. Hirsch, F.E.W. Eckhardt and R.J. Jr. Palmer, J. Microbiol. Methods **23**, 143(1995).
10. W.R. McKinney, C.J. Hirschmugl, H.A. Padmore, T. Lauritzen, N. Andresen, G. Andronaco, R. Patton, and M. Fong, Proceedings of the SPIE, Accelerator-Based Infrared Sources and Applications, **3153**, pp 59-67.
11. Michael C. Martin and Wayne R. McKinney, this proceeding volume.
12. K. Brandenburg and U. Seydel in Infrared Spectroscopy of Biomolecules, edited by H.H Henry and D. Chapman (Wiley-Liss, N.Y., 1996), p. 203-238.
13. J.A. Campbell, Spectrochimica Acta, **21**, 1333(1965).
14. D. Grant, W.F. Long, and F.B. Williamson, Biochem. Soc. Trans. **18**, 1281(1990)
15. J.A. Campbell, Spectrochimica Acta, **21**, 851(1965).
16. H.-Y.N. Holman and J.C. Hunter-Cevera, manuscript in preparation.